

U.S.S.N.: 09/403,690
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21. (Amended) A method for detection and differentiation of pathogenic enterobacteria in a sample, said method comprising:

isolating nucleic acid from said sample;

adding a set of oligonucleotide primer pairs to said sample, wherein said set of oligonucleotide primers comprises at least five oligonucleotide primer pairs, wherein at least one primer pair is capable of specifically amplifying a DNA sequence to produce an amplified product of a virulence factor/toxin gene characteristic for each one of an enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic or enterohemorrhagic pathogenic E. coli strain;

subjecting said sample and said set of primer pairs to an amplification process; and

detecting the presence of at least one amplified product, wherein the presence of at least one amplified product indicates the presence of at least one pathogenic enterobacteria strain in said sample.

22. (Amended) The method according to claim 21, wherein the set of oligonucleotide primer pairs comprises primer pairs selected from the group consisting of:

at least one primer pair that hybridizes to a gene encoding heat labile toxin or a gene encoding heat stable toxin for amplification of a DNA sequence characteristic for enterotoxigenic E. coli;

at least one primer pair that hybridizes to a gene encoding heat stable toxin or to a pCDVD432 plasmid for amplification of a DNA sequence characteristic for enteroaggregative E. coli;

at least one primer pair that hybridizes to a *inv*-plasmid for amplification of a DNA sequence characteristic for enteroinvasive E. coli;

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E1
at least one primer pair that hybridizes to a EAF plasmid, or an *eae* gene for amplification of a DNA sequence characteristic for enteropathogenic *E. coli*; and

at least one primer pair that hybridizes to the genes encoding shiga-like toxin *stxI* or *stxII* for amplification of a DNA sequence characteristic for enterohemorrhagic *E. coli*.

E2
24. (Amended) The method according to claim 21, wherein detecting the presence of at least one amplified product is performed using at least one oligonucleotide probe capable of hybridizing to the amplified product wherein said oligonucleotide probe is labeled at the 5' end with a fluorescent reporter dye and at the 3' end with a fluorescent quencher dye and is susceptible to 5'-3' exonuclease degradation by a polymerase, and wherein said PCR amplification process uses a polymerase having 5'-3' exonuclease degradation activity.

E3
26. (Amended) The method according to claim 24 wherein the labeled oligonucleotide probe is selected from the group consisting of:

a labeled oligonucleotide probe specific for the detection of a heat labile toxin gene characteristic for enterotoxigenic *E. Coli*;

a labeled oligonucleotide probe specific for the detection of a heat stable toxin gene characteristic for enterotoxigenic *E. Coli*;

a labeled oligonucleotide probe specific for the detection of a heat stable toxin gene characteristic for enteroaggregative *E. Coli*;

a labeled oligonucleotide probe specific for the detection of a pCVD432 plasmid;

a labeled oligonucleotide probe specific for the detection of a *inv*-plasmid;

a labeled oligonucleotide probe specific for the detection of a EAF-plasmid;

a labeled oligonucleotide probe specific for the detection of a *eae* gene;

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a labeled oligonucleotide probe specific for the detection of a shiga-like toxin SltI gene;
and

a labeled oligonucleotide probe specific for the detection of a shiga-like toxin SltII gene.

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29. (Amended) The method according to claim 21 wherein the amplification process comprises 35 PCR cycles at a MgCl₂ concentration of 5.2 mM, an annealing temperature of 55 °C and an extension temperature of 65 °C.

30. (Amended) A set of oligonucleotide primer pairs useful for polymerase chain reaction (PCR) amplification of DNA of pathogenic enterobacteria allowing detection and differentiation of pathogenic enterobacteria in a sample wherein following amplification the presence of at least one amplified product indicates the presence of at least one pathogenic enterobacteria strain in said sample, wherein said set comprises at least five primer pairs, wherein each primer pair specifically amplifies a DNA sequence of a virulence factor/toxin gene characteristic for one each of the subgroups of the pathogenic E. coli strains, said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic E. coli strains and wherein for amplification of each subgroup at least one oligonucleotide primer pair is included in said set of oligonucleotide primer pairs.

31. (Amended) The set of primer pairs according to claim 30 comprising

a primer pair that hybridizes to a gene encoding heat labile toxin, or to a gene encoding heat stable toxin of enterotoxigenic E. coli;

a primer pair that hybridizes to a gene encoding heat stable toxin or to a pCVD432 plasmid of enteroaggregative E. coli;

a primer pair that hybridizes to a inv-plasmid of enteroinvasive E. coli;

a primer pair that hybridizes to a EAF plasmid, or a eae gene of enteropathogenic E. coli;
and

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24
a primer pair that hybridizes to a gene encoding shiga-like toxin stII or stIII of enterohemorrhagic E. coli.

25
33. (Amended) A set of labeled oligonucleotide probes useful for detection and differentiation of pathogenic enterobacteria in a sample by Real Time-PCR, each probe specifically binding a sequence of a virulence factor/toxin genes characteristic of one of the subgroups of pathogenic E. coli strains comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic E. coli strains, wherein said oligonucleotide probes are labeled at the 5' end with a fluorescent reporter dye and at the 3' end with a fluorescent quencher dye and are susceptible to 5'-3' exonuclease degradation by a polymerase, and wherein for detection and differentiation of each subgroup at least one probe is included in the set of oligonucleotide probes.

34. (Amended) The set of probes according to claim 33 comprising:

a labeled oligonucleotide probe specific for the detection of heat labile toxin gene characteristic for enterotoxigenic E. Coli or a labeled oligonucleotide probe specific for the detection of heat stable toxin gene characteristic for enterotoxigenic E. Coli;

a labeled oligonucleotide probe specific for the detection of heat stable toxin gene characteristic for enteroaggregative E. Coli or a labeled oligonucleotide probe specific for the detection of pCVD432 plasmid;

a labeled oligonucleotide probe specific for the detection of the inv-plasmid;

a labeled oligonucleotide probe specific for the detection of the EAF-plasmid or a labeled oligonucleotide probe specific for the detection of the eae gene; and

a labeled oligonucleotide probe specific for the detection of shiga-like toxin StII gene or a labeled oligonucleotide probe specific for the detection of shiga-like toxin StIII gene.

26
36. (Amended) A set of oligonucleotide primer pairs and a set of oligonucleotide

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primer probes useful for diagnosing an enterobacteria infection in samples derived from a living animal body including a human, by Real time PCR method, wherein said sets of oligonucleotide primer pairs and oligonucleotide primer probes allow detection and differentiation of pathogenic enterobacteria in a sample, wherein said set of oligonucleotide primer pairs comprises at least five primer pairs and at least one primer pair specifically amplifies a DNA sequence of a virulence factor/toxin gene characteristic for each of the subgroups of the pathogenic E. coli strains, said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic E. coli strains and, wherein said set of oligonucleotide probes comprises at least one oligonucleotide probe, each oligonucleotide probe specifically binding a sequence of a virulence factor/toxin genes characteristic of one of the subgroups of pathogenic E. coli strains, said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic E. coli strains by real time PCR.

EX
38. (Amended) The method of claim 37, wherein said sample is derived from a human.

E8
40. (Amended) The method of claim 39, wherein said consumable is selected from the group consisting of meat, milk and vegetable.

REMARKS

STATUS OF THE CLAIMS

Claims 21-40 were pending in this application. Claim 25 has been cancelled without prejudice or disclaimer. Claims 21, 22, 24, 26, 29, 30, 31, 33, 34, 36, 38, and 40 have been amended. The amendments to the claims and the cancellation of claim 25 is not intended to be a dedication to the public of the subject of the claims as previously presented. Applicant reserves the right to file the same or similar claims pursuant to 35 U.S.C. § 120.

A marked up version of the amended claims is provided in Appendix I. Following entry of the amendments claims 21-24 and 26-40 will be pending and at issue. A clean copy of all